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# The Effect of Natural Antioxidants on CLA Yield during the Photoisomerization of Soy Oil Linoleic Acid

Submitted in partial fulfillment of the requirements for the  
University of Arkansas Dale Bumpers College of Agricultural, Food and Life Sciences  
Honors Program

By

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April 23, 2012  
University of Arkansas

## ABSTRACT

Dietary conjugated linoleic acid (CLA) is known to be effective in avoiding many obesity related diseases. Conjugated linoleic acid is a product of ruminant fermentation and 3.4g/day are needed to obtain the clinical benefits. However, it is difficult to obtain sufficient CLA to realize these benefits from a healthy diet containing dairy and beef products, without increasing levels of dietary cholesterol and saturated fat. A 20% CLA soy oil with low saturated fat and no cholesterol has been produced by the photoisomerization of linoleic acid in the triacylglyceride oil. Further increasing the CLA yields has been possible by addition of tocopherol antioxidants. The objectives of this research were to determine the effects of other natural phenolic antioxidants on CLA yield and oxidative stability during photoisomerization. Rosemary extract (RME), rosmarinic acid (RA), gallic acid (GA), caffeic acid (CA), and chlorogenic acid (CHA) were each added to refined bleached deodorized soy oil at levels they were reported to serve best as an antioxidant. The oil was then photoisomerized to produce CLA-rich oil. The CLA levels in soy oil were determined by gas chromatography - flame ionization detector (GC-FID) as fatty acid methyl esters (FAMES). The oxidative stability was determined by peroxide value (PV). The order of effectiveness as a CLA promoter was  $CHA > RME > RA > CA > GA$ . CHA at 11 ppm showed the greatest increase in CLA yield and a much lower PV than the control. RME was less effective than CHA while the CA, GA and RA were ineffective. A balance of polarity/non-polarity and antioxidant concentration seem to be the most important factors in determining CLA yields, oil solubility and antioxidant performance.

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## INTRODUCTION

Conjugated linoleic acid (CLA) isomers and dietary 9,12 linoleic acid (LA) are both octadienoic acids but in CLA the diene structure is conjugated and can be either *cis* 9, *trans* 11 or *trans* 10, *cis* 12 whereas linoleic acid has *cis* 9, *cis* 12 methylene-interrupted double bonds producing very different isomers. CLA was discovered as a product of ruminant fermentation and found in beef and dairy products in the 1930's, but it was not until the 1980's that its potential health benefits were discovered in *in vitro* and animal studies. These CLA health benefits include anti-carcinogen (Ip *et al.* 1994), anti-obesity (West *et al.* 1998), and anti-diabetic activity effects (Houseknecht *et al.* 1998). Unfortunately, the average daily CLA consumption is not sufficient to obtain the recommended 3.4g of CLA necessary to realize the associated health benefits (Ip *et al.* 1994). This is because fat in beef and dairy products contain low levels of CLA at 0.3 - 0.8%. If an increased CLA intake was achieved through increased dietary bovine and dairy products, there would be a corresponding increase in the consumption of saturated fats and cholesterol. This would be undesirable, as saturated fats and cholesterol increase the risk of cardiovascular disease and of cancer. Therefore, alternative ways to obtain high levels of dietary CLA from a low saturated fat, low cholesterol food source would be helpful.

Conjugated linoleic acid may be produced directly from linoleic acid through fermentation (Martin and Jenkins 2002; Vahvaselka *et al.* 2004; Lin *et al.* 2005) and organic synthesis (Yang and Liu 2004). However, these methods are time consuming, produce low CLA yields, are expensive, tedious and are not commercially viable. Soy oil is an ideal candidate as a source of CLA as it contains 50% LA, is low in saturated fats (<10%), naturally contains no cholesterol and is the most common, inexpensive vegetable oil in the US. Jain *et al.* (2008a) developed a simple method to photoisomerize soy oil linoleic acid to CLA to produce 20% CLA rich oil. This was done in a pilot plant setting requiring only elemental iodine and 12 hours UV/vis light. The iodine was then removed by either adsorption or distillation. Jain *et al.* (2008b) showed that the higher the degree of oil processing in this process i.e. the more minor crude oil components were removed, the greater the CLA yields. Tokle *et al.* (2009) investigated the effect of each minor crude oil component on soy oil CLA yields and found that free fatty acids (FFA), peroxide oxidation products and phospholipids all decreased CLA yields with peroxide oxidation products having the greatest effect. Lutein and free fatty acids had very little effect on CLA yield whereas tocopherols, a soy oil antioxidant, increased CLA yields. In a subsequent study, Yetella *et al.*

(2011) showed that adding 1400 ppm of mixed tocopherols significantly increased CLA yields while also decreasing peroxide values, which indicates greater oil oxidative stability.

The goal of this research is to investigate the role of selected natural phenolic antioxidants such as chlorogenic acid, rosemary extract, rosmarinic acid, caffeic acid and gallic acid in promoting CLA formation during photoisomerization of soy oil linoleic acid and in protecting soy oil against oxidation.

The specific objective of this study was to:

- 1) Determine the effect of chlorogenic acid, rosemary extract, rosmarinic acid, caffeic acid and gallic acid on CLA yield during the photoisomerization of soy oil linoleic acid
- 2) Determine the effect of chlorogenic acid, rosemary extract, rosmarinic acid, caffeic acid and gallic acid on oxidative stability of CLARSO during photoisomerization



## LITERATURE REVIEW

### CLA health effects

*Anti-carcinogenic effects:* Pariza and Hargraves found an antimutagen in ground beef in 1985 (Pariza and Hargraves 1985). Later, this antimutagen was found to be CLA (Ha *et al.* 1987). Mice that were treated with CLA showed a slower tumor growth. In a later study, rats were induced with mammary tumors and were then given diets with different percentages of CLA based on their weights. The results showed a decrease in tumor size by 50% when a 0.5% CLA diet was introduced. Very few human studies are available on the anti-carcinogenic effects of CLA but there have been studies to examine CLA and its correlation with breast cancer. Aro *et al.* (2000) discovered that women who consumed CLA regularly had a 60% less chance of developing breast cancer as compared to those who did not regularly consume CLA.

*Anti-obesity/Body composition/Anti-diabetic effects:* Studies to determine CLA's effect on body composition have been misleading. The studies conducted with mice have shown that diets supplemented with CLA have decreased fat content and increased protein content. In the mice, the amount of fat in the mice's diet didn't have an effect on the CLA's effects. Low and high fat diets both showed weight reductions (West *et al.* 1998). The studies conducted on humans have shown that CLA did and did not have an effect. A recent study found that adding 3.4g of CLA did not have an effect on the subjects' body weight (Larsen *et al.* 2006). On the other hand, humans that added 0.7g of CLA for 4 weeks and then 1.4g of CLA for another 4 weeks showed decreases in skin fold thickness, body fat percentages, and fat mass (Mougios *et al.* 2001). This discrepancy could be due to the effects of different CLA isomers and the dosages that make them effective that have yet to be determined. CLA's anti-diabetic properties have shown to improve glucose tolerance and fight hyperinsulinemia in diabetic rats as effectively as current medications (McGuire and McGuire 1999). Another study showed that CLA acts to sensitize the body to insulin, lowering insulin resistance, therefore lowering total insulin levels (Riserus *et. al.* 2002).

### CLA synthesis from soy oil

*Photoisomerization of soy oil linoleic acid.* Soybean oil contains 50% linoleic acid, is low in saturated fats and contains no cholesterol. Thus, soybean oil is highly suitable for the production of CLA rich oil. It was shown by Jain and Proctor (2006) that CLA could be produced by the photoisomerization of linoleic acid in soybean oil

using an iodine catalyst and UV light in a laboratory setting. Different concentrations of iodine (0, 0.1, 0.25, and 0.5%) were added to 200g of RBD soy oil. This was placed with a 100W mercury lamp above the oil for 120h. The control, with no iodine present, produced no CLA, which indicated the importance of the iodine as a catalyst. The UV lamp was placed too high above the oil to properly penetrate it and produce any significant amount of CLA (0.6% CLA after 90h).

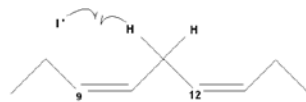
Jain and Proctor (2008) enhanced the process by developing a pilot plant scale irradiation unit in order to maximize CLA yield, minimize processing time and eliminate iodine from the final product. They created an illuminated laminar flow unit (ILFU) made up of borosilicate glass plates and three 450W UV/visible lamps. This allows for maximum exposure of the oil to the UV light, which was shown to increase CLA production almost linearly. Because of the thin layer of oil, processing time was also greatly reduced to 12h. Iodine adsorption using bleached clay significantly decreased the amount of iodine in the final irradiated product. The development of this method is significant in that CLA-rich soy oil can be inexpensively made and edible for human consumption.

*Antioxidants effects on CLA yield:* Studies have shown that low levels of tocopherols increased CLA yields during the photoisomerization of linoleic acid in soy oil (Tokle *et al*, 2009). In a recent study, mixed tocopherols, TBHQ, and  $\gamma$  tocopherols were used to increase CLA yields and reduce formation of lipid oxidation products during the photoisomerization of soy oil linoleic acid (Yettella *et al*, 2011).

*Mechanism of CLA formation by photoisomerization:* Figure 1 shows the proposed mechanism by which *trans* 10, *cis* 12 CLA is formed by a free radical mechanism in soybean oil. However, *cis* 9, *trans* 11 CLA isomer forms by the same reaction. Molecular iodine is cleaved into two free radical iodine molecules by UV light. The iodine free radical abstracts a hydrogen from the methylene carbon on linoleic acid. The resulting lipid free radical is stabilized by migration of a double bond to achieve a conjugated diene structure and thus creates a conjugated system. The hydrogen, initially abstracted by the iodine radical to form HI, binds with the newly positioned carbon radical, producing a iodine radical. Because the radical can occur at C<sub>9</sub> or C<sub>13</sub>, multiple CLA isomers will form. This process generates *cis* 9, *trans* 11 or *trans* 10, *cis* 12 CLA isomer. A further reaction can occur whereby the bound iodine allows free rotation around the single carbon-carbon bond adjacent to the carbon-iodine bond. This allows formation of the *trans*, *trans* diene, after loss of iodine. In contrast to biological

sources of CLA, *trans, trans* isomers formed are the most common CLA isomers in soy oil by this method.

**Figure 1 – Free radical mechanism of LA isomerization to CLA with an iodine catalyst.**

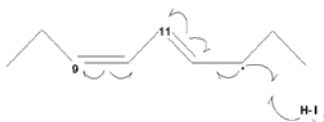


LA (*cis*-9, *cis*-12 octadecadienoic acid)

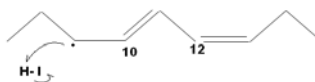
*Radiation cleaves I<sub>2</sub> and I<sup>•</sup> abstracts H-atom from C-11 to form a radical.*



*Resonance structure creates new carbon radical*

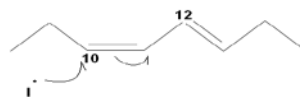


*Hydrogen atom from HI comes together with the C-13 radical.*



*trans*-10, *cis*-12 CLA

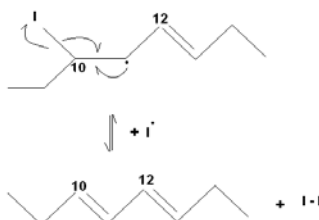
*H-atom from HI binds with C-9, I<sup>•</sup>'s from HI bind to form I<sub>2</sub>.*



*I<sup>•</sup> adds to C-10 creating a trans product.*



*Addition of I<sup>•</sup> allows free rotation around C-10.*



*trans*-10, *trans*-12 CLA

*I<sup>•</sup> abstracts I<sup>•</sup> to give the product.*

## MATERIALS & METHODS

### Materials:

Refined, bleached, and deodorized (RBD) soy oil was obtained from Riceland Foods (Stuttgart, AR) and used as the control. Resublimed iodine crystals (EM Science, Cherry Hill, NJ) were used as a catalyst. Commercial CLA methyl esters (Sigma-Aldrich, St.Louis, MO) containing a mixture of *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA, and *trans*-,*trans*-CLA isomers were used as a standard and heptadecanoic acid methyl ester (17:0; Sigma-Aldrich) was used as the internal standard. Sodium methoxide and anhydrous sodium sulfate (EMD Chemicals, Darmstadt, Germany) was used for methyl ester preparation. Magnesol<sup>®</sup>, commercial magnesium silicate was obtained from The Dallas Group of America, Inc. (Whitehouse, NJ). Helium, air and hydrogen gas were obtained from Scientific Supplies (University of Arkansas, Fayetteville, AR). Chlorogenic acid, caffeic acid, rosmarinic acid, and gallic acid were obtained from Sigma-Aldrich (St.Louis, MO) and rosemary extract was obtained from Danisco (Copenhagen, Denmark).

### Methods:

#### *PROCESSING*

*Pretreatment of soy oil.* Five percent Magnesol<sup>®</sup> magnesium silicate adsorbent, was added to 800g of refined bleached deodorized (RBD) soy oil and mixed for 15 minutes using a magnetic stirrer to remove oxidation products that would reduce CLA yields. The oil was then vacuum filtered, deaerated with a sonicator for 30 min and placed in a 1L beaker wrapped with aluminum foil to prevent exposure of oil to light.

*Iodine and antioxidant addition to the oil.* Oil was heated to 70 °C while flushing with nitrogen to avoid oxidation and 0.35% iodine was added to the oil. The oil was then stirred until the iodine was completely dissolved and allowed to cool to room temperature (Jain and Proctor, 2006). One hundred gram aliquots of a range of concentrations of chlorogenic acid, caffeic acid, gallic acid, rosmarinic acid, and rosemary extract were prepared as shown in Figure 1. The selected concentration range of each antioxidant was based on the concentration range they were found to be most effective as an antioxidant. Duplicate 5ml samples were pipetted into 7ml borosilicate vials for photoirradiation. Duplicate control samples of oil without added antioxidant were included with each treatment.

*Photoisomerization.* These vials were placed on a photoirradiation unit in areas to facilitate maximum, uniform UV light exposure and irradiated for 12 hours as described by Jain *et al.* 2008b and Lall *et al.* 2009.

**Table 1 – Antioxidants and their concentrations used in this study**

ANTIOXIDANT	CONCENTRATIONS (PPM)	CITATION
Caffeic Acid (CA)	9, 18, 27, 36	(Chen and Ho, 1997)
Chlorogenic Acid (CHA)	11, 14, 18, 71, 106	(Laguerre <i>et al.</i> , 2011)
Gallic Acid (GA)	8.5, 17, 25.5, 34	(Frankel and Huang, 1997)
Rosmarinic Acid (RA)	40, 50, 60, 100	(Aeschbach <i>et al.</i> , 1996)
Rosemary Extract (RME)	300, 400, 500, 600	(Frankel and Huang, 1996)

#### *OIL ANALYSIS*

Each duplicate oil sample was subjected to fatty acid analysis as fatty acid methyl esters (FAMES) by gas chromatography - flame ionization detection (GC-FID) to determine the CLA content. Peroxide value (PV) analysis was conducted to determine oil oxidative stability after processing. Each duplicate sample was subjected to duplicate analysis for each method.

*Fatty acid methyl ester (FAMES) formation for GC-FID analysis.* One hundred mg of photoisomerized soybean oil was weighed into a 25 mL centrifuge tube, and 500 µL of 1% heptadecanoic acid methyl ester (17:0, internal standard), 2 mL of toluene, and 4 mL of 0.5 M sodium methoxide in methanol were added to the centrifuge tube and then purged with nitrogen gas. The centrifuge tube was heated to 50 °C for 10 min and then cooled for 5 min. After the tube had cooled, 200 µL of glacial acetic acid was added to the centrifuge tube to prevent the formation of sodium hydroxide. Five mL of distilled water was added to the centrifuge tube followed by 5 mL of hexane, and the tube was vortexed for 2 min. The hexane layer was extracted and dried over anhydrous sodium sulfate in a 7 mL glass vial (Christie 2003). The extracted layer was then taken from the glass vial and placed in a gas chromatograph vial. Methyl esters were analyzed by gas chromatography (GC) using an SP 2560 fused

silica capillary column (100 m x 0.25 mm i.d. x 0.2 µm film thickness; Supelco Inc., Bellefonte, PA) (Ma *et al.* 1999) with a flame ionization detector (FID) (model 3800, Varian, Walton Creek, CA). Duplicate 2 µL samples, prepared in hexane, were injected by an autosampler CP8400 (Varian), and gas chromatograms were collected by Galaxie Chromatography Workstation 1.9.3.2 (Varian). Two determinations each consisting of duplicate injections were conducted for each treatment. CLA concentrations were calculated by the following equation:

$$\text{Isomer conc.} = \frac{[\text{internal standard conc. (5mg)} \times \text{peak area} \times \text{relative response factor}]}{\text{internal standard peak area}}$$

*Peroxide Value Analysis.* Peroxide values (PV) of the photoisomerized samples were measured in duplicate according to an AOCS acetic acid-choloroform method (AOCS Cd 8-53).

*Statistical Analysis.* All samples were prepared in duplicate and duplicate analysis of each sample was done. Analysis of variance (ANOVA) was conducted on all data using JMP version 5.0.1 (SAS Institute Inc., Cary, NC). A student's *t* test was used to differentiate mean values, with significance defined at  $p < 0.05$ . Standard deviations were also determined.

## RESULTS AND DISCUSSION

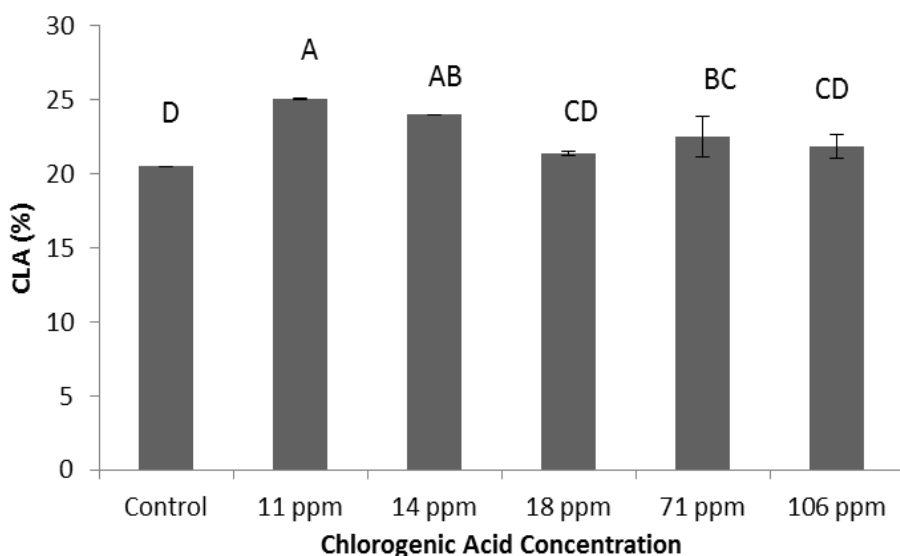
*Chlorogenic acid (CHA):* Figure 2a shows the effect that various chlorogenic acid (CHA) concentrations had on soy oil total CLA yields, relative to the control. CHA levels of 11, 14 and 71 ppm produced CLA levels of 25.1%, 24%, and 22.5% respectively, which was significantly greater than the level of 20.5% found in the control.

The CLA isomers content is seen in Figure 2b. The *trans, trans* isomer comprises the majority of the CLA. This is reported in all previous CLA-rich oil studies as it is the most thermodynamically stable form. The *cis, trans* and *trans, cis* isomers each make up approximately 1-2% of the total CLA produced. Although the *trans,trans* isomer is synthesized from the *cis,trans* configurations, trans geometry has a lower energy and is thus more stable as compared to any cis configuration.

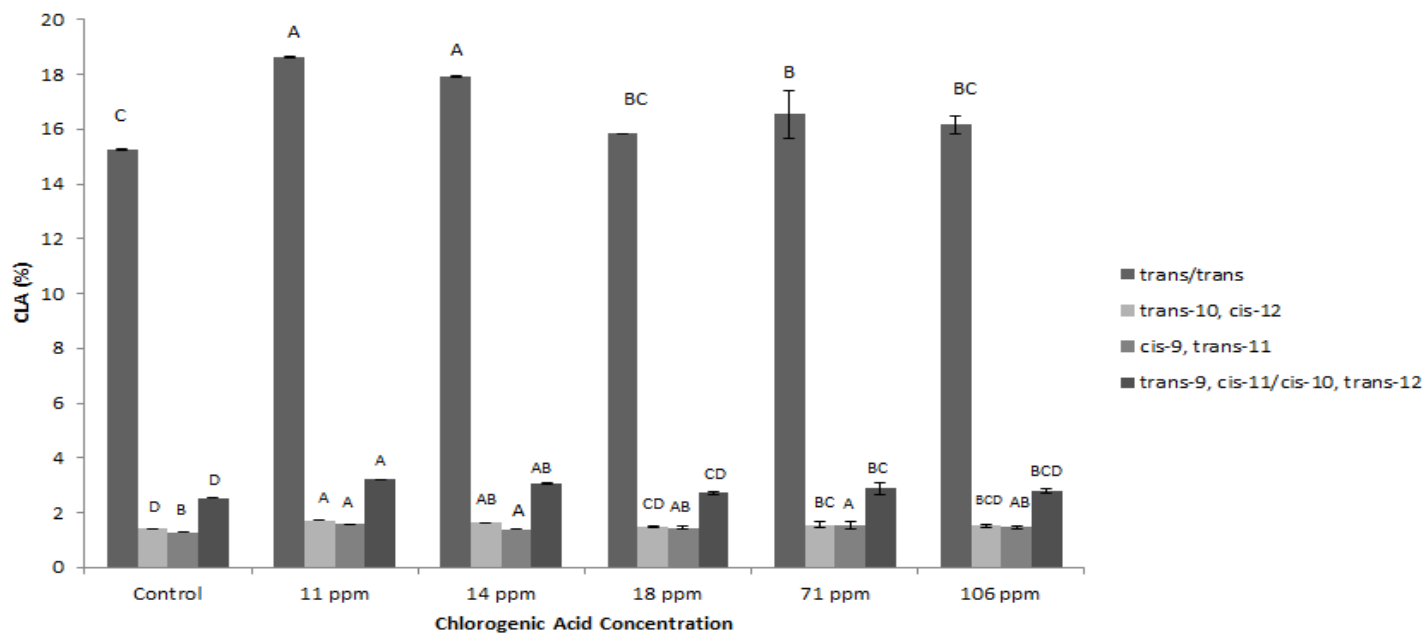
Figure 2c shows the effect of CHA on the peroxide value. All oil CHA concentrations had statistically significant lower PV relative to the control. Treatments producing the greatest antioxidant effect appear to also

produce the greatest CLA levels. Thus, CHA would be a viable additive to increase the CLA content of CLA-rich oil.

**Figure 2a – Effect of chlorogenic acid concentration on total CLA yield in refined, bleached, deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**

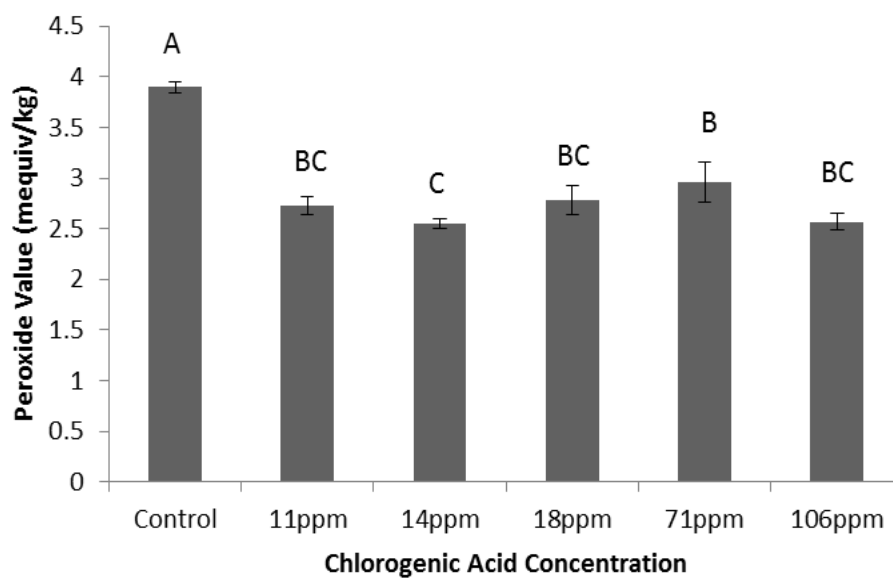


**Figure 2b – Effect of chlorogenic acid concentration on CLA isomer distribution in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**





**Figure 2c - Effect of chlorogenic acid concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



*Rosemary extract (RME)*: Figure 3a shows the effect that various RME concentrations had on total CLA in soy oil. The RME levels of 300, 500, and 600 ppm produced a small but significant increase in CLA of 18.7%, 18.1%, and 19.4% respectively, relative to the control value of 17.8%. The 400 ppm RME level significantly reduced total CLA yield producing only 13.2% CLA. The CLA isomers levels shown in Figure 3c are typical of those found in isomerization of soy oil linoleic acid.

Only RME at 300ppm had a statistically significant effect in reducing the PV during processing, relative to the control. Oil PV of oil with 400ppm and 500 ppm RME were not significantly different from that of the control, while 600 ppm produced a greater PV.

*Rosmarinic acid (RA)*: The effect that various concentrations of RA had on total CLA in soy oil is seen in Figure 4a. None of the treatments produced an increase in CLA relative to the control level of 21.8%. The 50 ppm level produced less CLA than the control and other treatments. This is reflected in the CLA isomer distribution (Figure 4b). Rosmarinic acid is hydrophilic and very water soluble. Its' hydrophilicity made solubilization in the soy oil difficult which may explain the results.

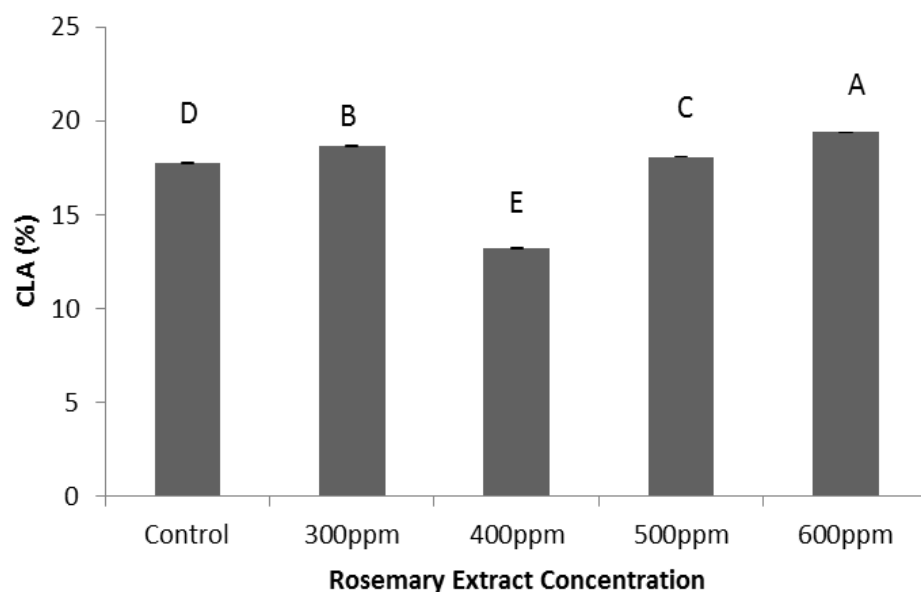
The PV data in Figure 4c shows that at lower RA levels there was no significant difference in PV, relative to the control. However, there was an elevated PV value at the higher RA concentrations of 60 ppm and 100 ppm. The greater PV at higher levels may be due to the greater mixing needed to achieve dissolved RA and thus the greater probability of incorporating oxygen into the oil.

*Caffeic acid (CA)*. None of the CA concentrations produced a statistically significant increase of CLA yield relative to the control. Significant decreases in CLA yield are observed at 9 ppm (25.16% CLA) and 36 ppm (24.94% CLA). These trends were also shown in the CLA isomer distribution (Figure 5b).

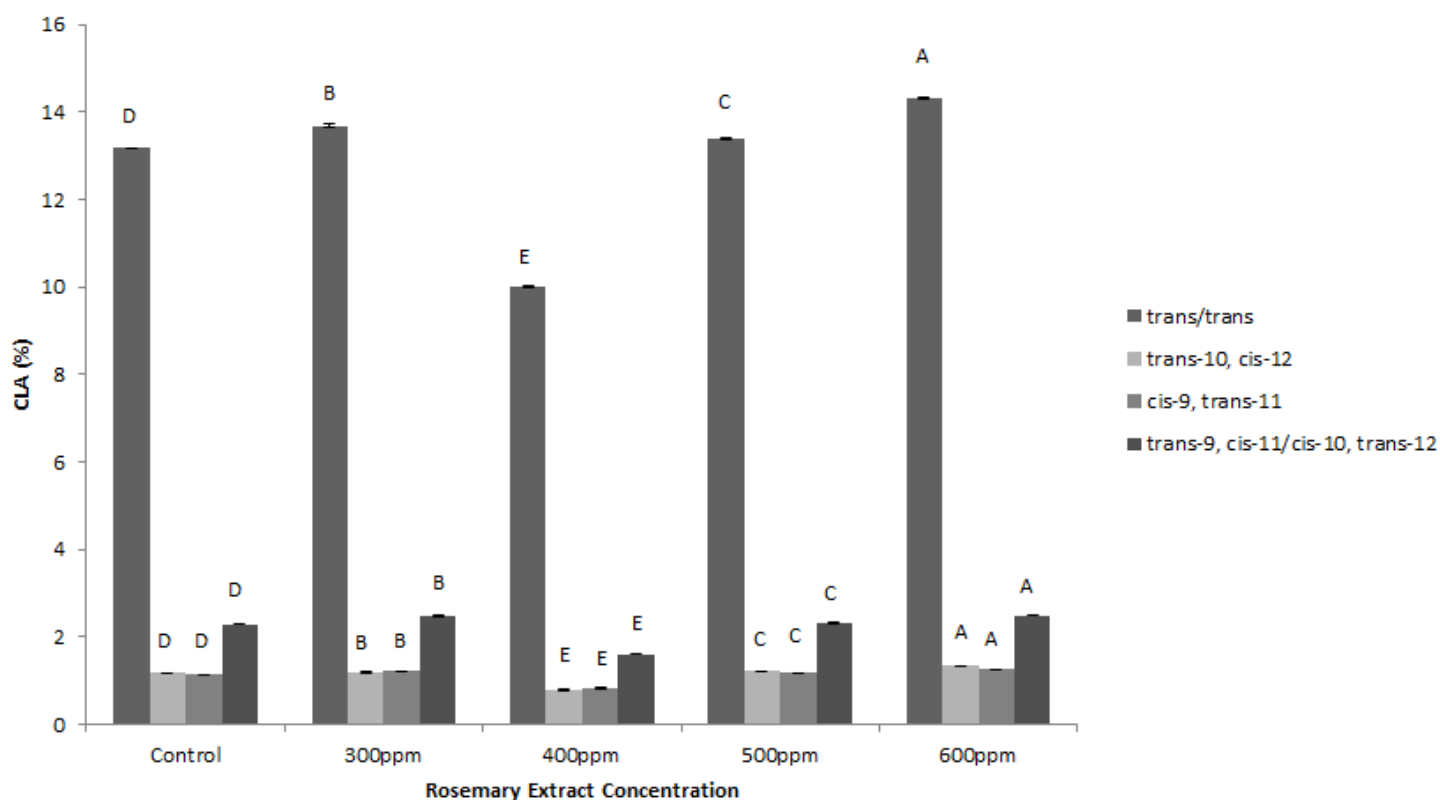
Figure 5c represents the PV at various concentrations of CA. All concentrations of CA produced a significantly higher PV relative to the control, which had a PV of 1.23 mequiv/kg.

Thus, the presence of CA seemed to not affect, or reduced, CLA yields while reducing oxidative stability at the concentrations used.

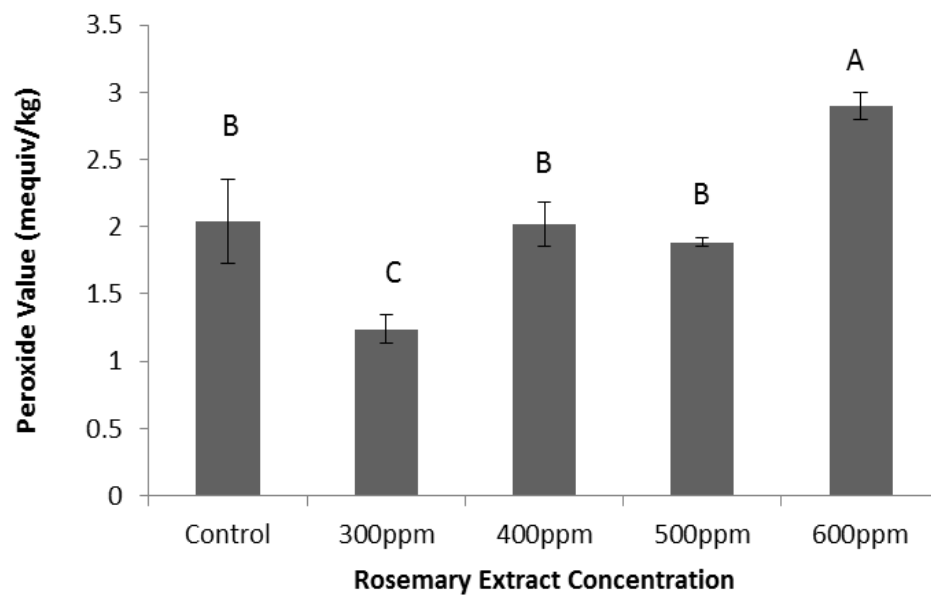
**Figure 3a - Effect of rosemary extract concentration on total CLA yield in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



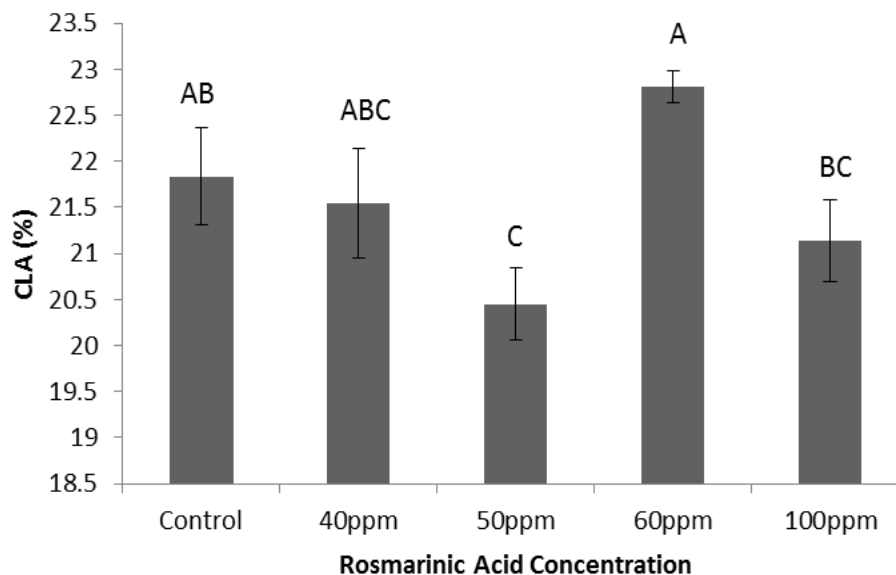
**Figure 3b - Effect of rosemary extract concentration on CLA isomer distribution in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



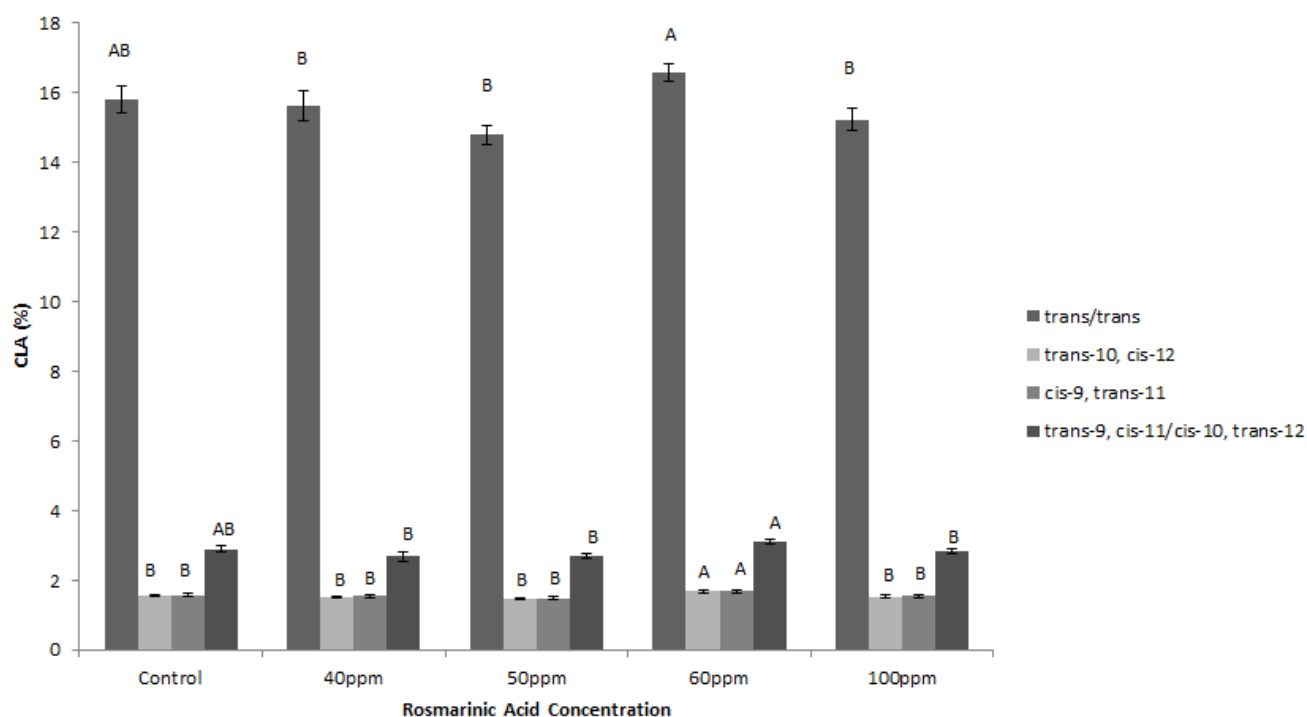
**Figure 3c - Effect of rosemary extract concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



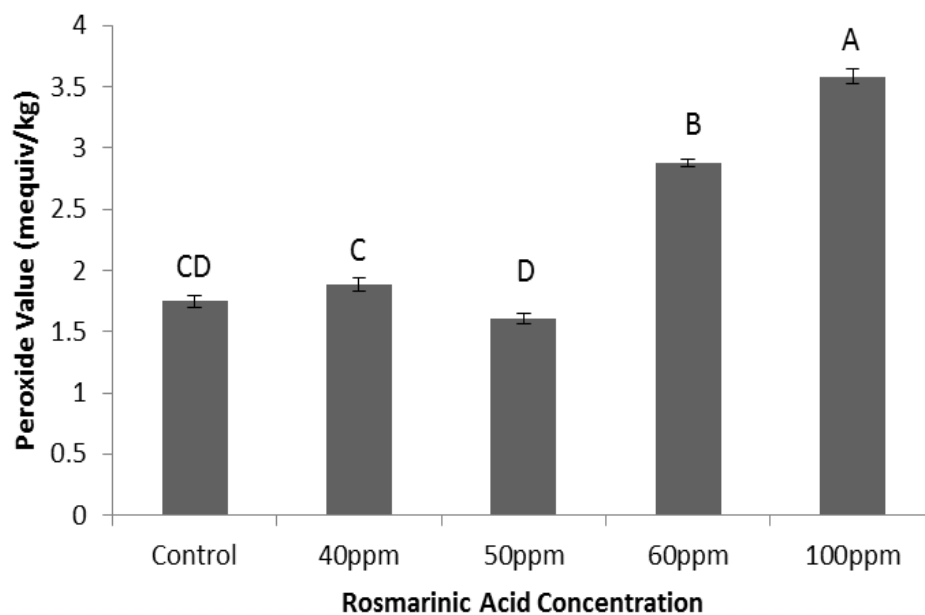
**Figure 4a - Effect of rosmarinic acid concentration on total CLA yield in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



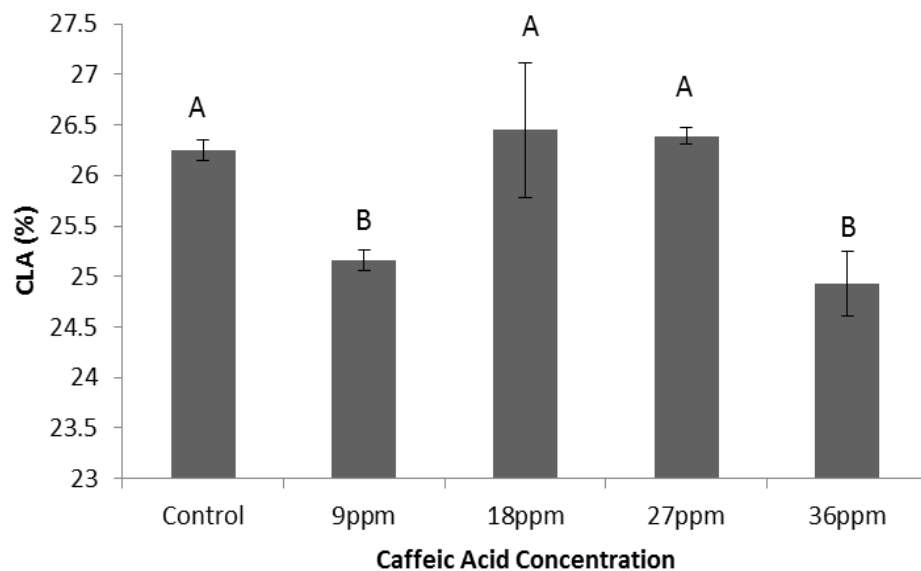
**Figure 4b - Effect of rosmarinic acid concentration on CLA isomer distribution in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



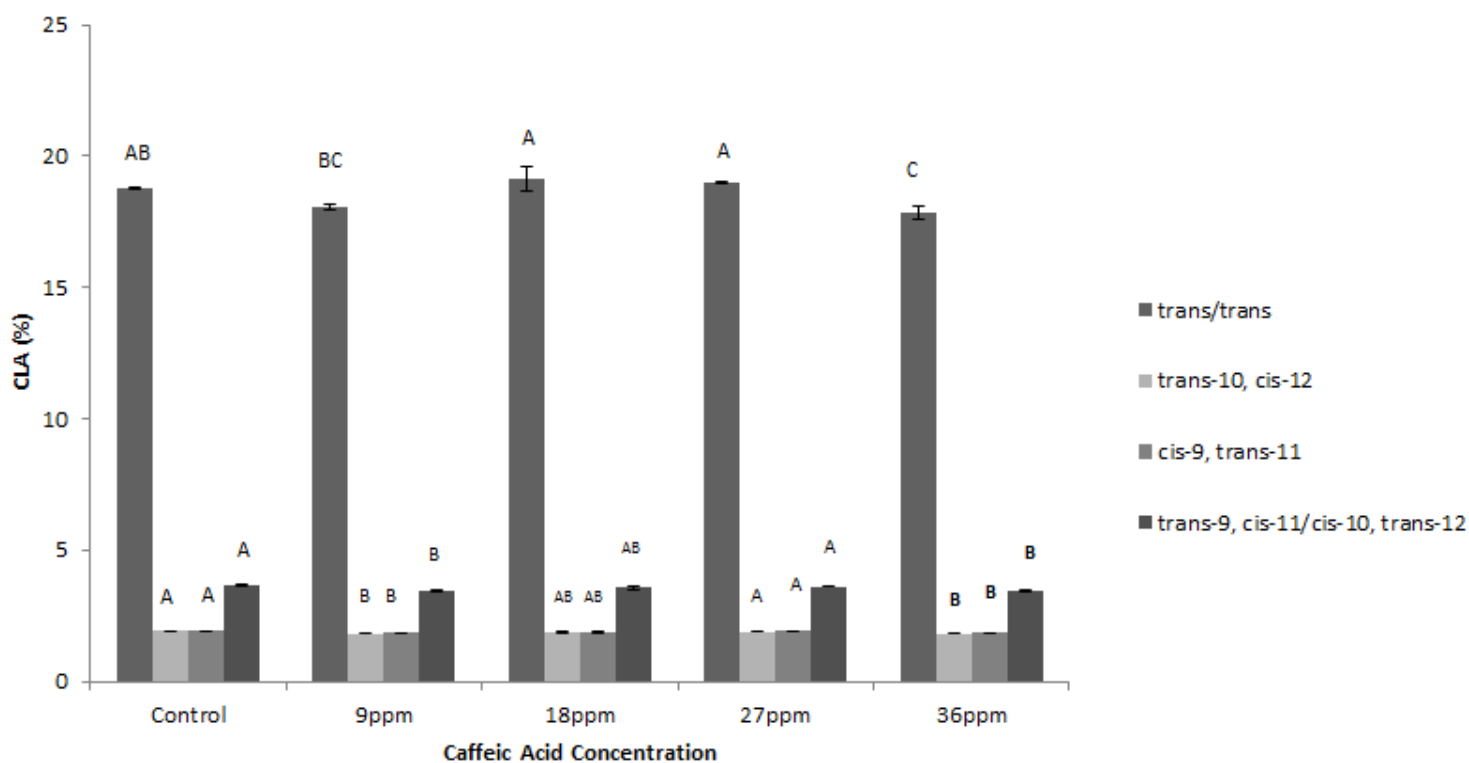
**Figure 4c - Effect of rosmarinic acid concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



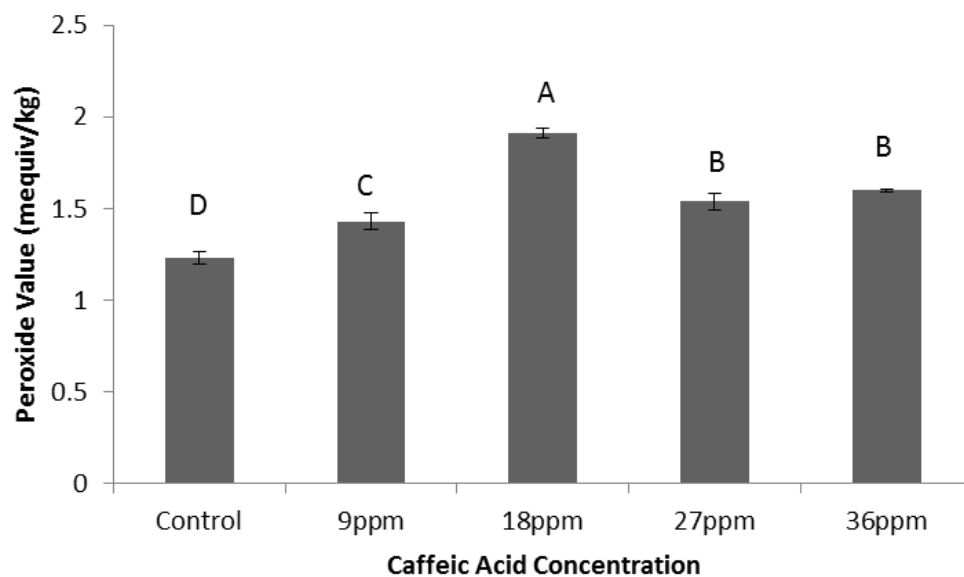
**Figure 5a - Effect of caffeic acid concentration on total CLA yield in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



**Figure 5b - Effect of caffeic acid concentration on CLA isomer distribution in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



**Figure 5c - Effect of caffeic acid concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**





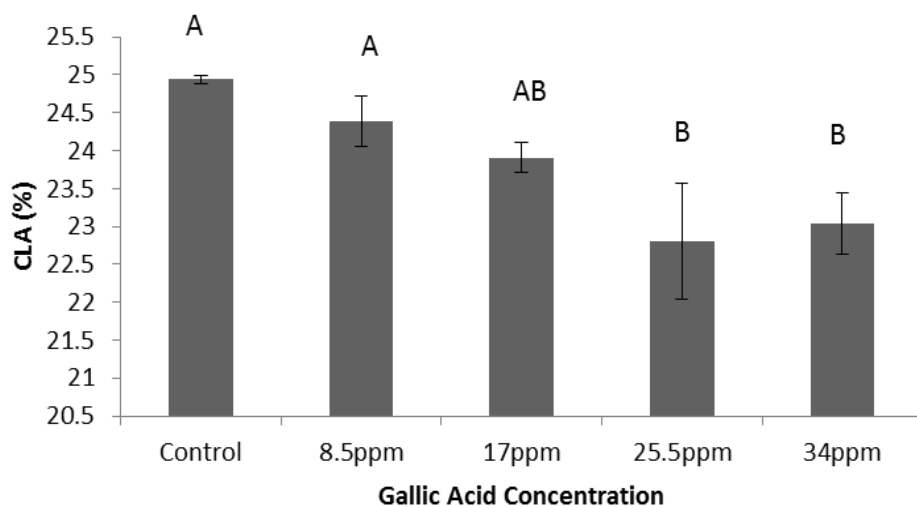
*Gallic acid (GA)*: Figure 6a shows the effect of GA on total CLA production. Increasing GA concentration at lower levels had no effect on CLA production, but inhibited production at higher levels relative to the control. This is also seen in the CLA isomer composition (Figure 6b).

Figure 6c shows the PV data for various concentrations of GA. The low GA levels did not protect against oxidation, relative to the control. Higher GA concentrations resulted in a significant increase in PV relative to the control.

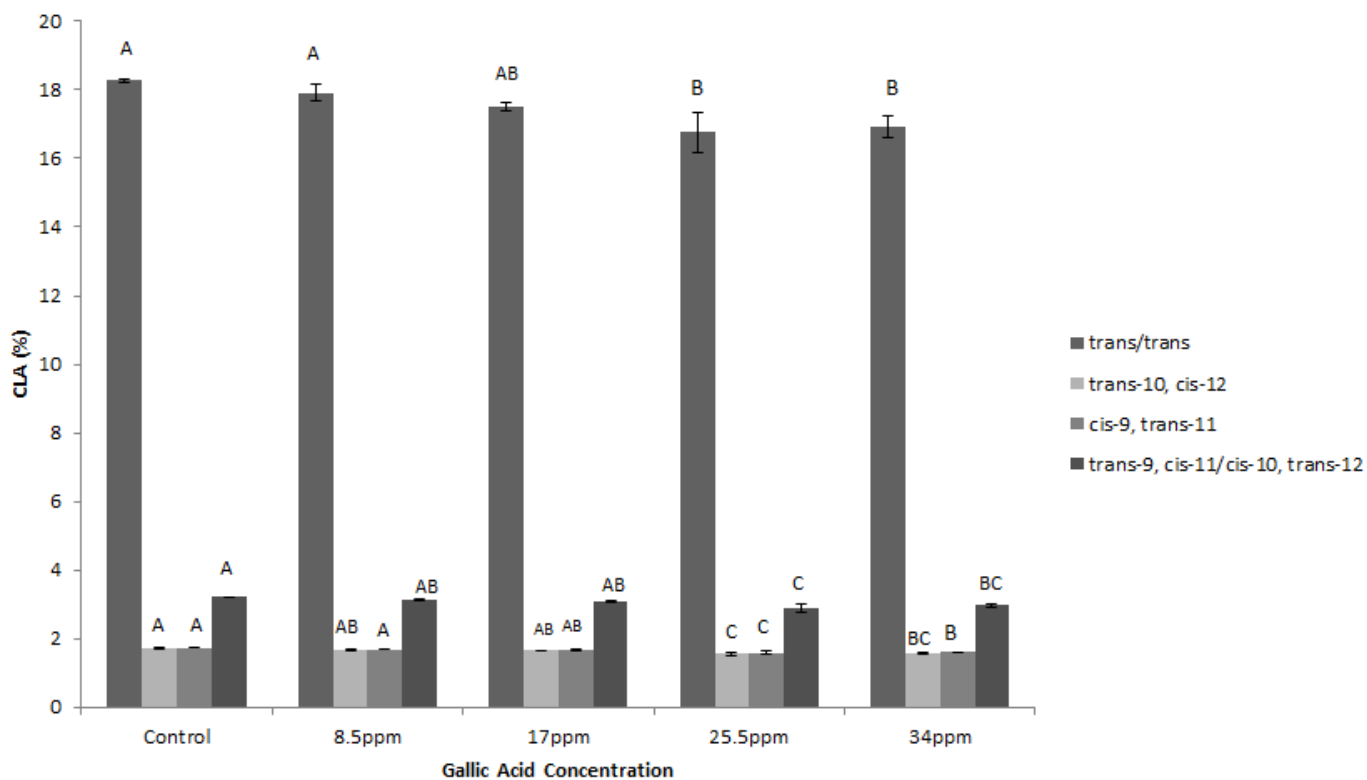
The effectiveness of the phenolic antioxidants were chlorogenic acid (11-106 ppm) > rosemary extract (300-600 ppm) > rosmarinic acid (40-100 ppm) > caffeic acid (9-36 ppm) > gallic acid (8.5-34 ppm), with only chlorogenic acid and rosemary extract increasing CLA yields. The most effective phenolics were used at higher concentrations relative to the other antioxidants. The concentration was an important factor. Therefore, the nature of the antioxidant should be considered (Figure 7). It has been suggested that the antioxidant concentration in oil is critical to its performance (Shahidi and Zhong, 2011). However, CHA at 11 ppm was the most effective treatment throughout. The literature values of optimum antioxidant concentrations did not pertain to the UV irradiation processing conditions.

In order for the antioxidant to be effective in CLA production it should be sufficiently non-polar to dissolve in oil, but in order to have antioxidant activity in oil it has to be polar enough to migrate to air-oil interface of microscopic air bubbles to serve as a radical scavenger. CHA seems to have both these characteristics to perform as a CLA promoter and antioxidant at the interface. Carnosic acid and carnosol in RME would seem to have these characteristics, but to a much lesser degree. In contrast, RA, CA and GA are more polar, therefore require more time to dissolve and thus greater possibility of mixing oxygen in the oil while stirring, even under a nitrogen blanket. Thus this would result in higher PV levels at higher RA, CA and GA levels.

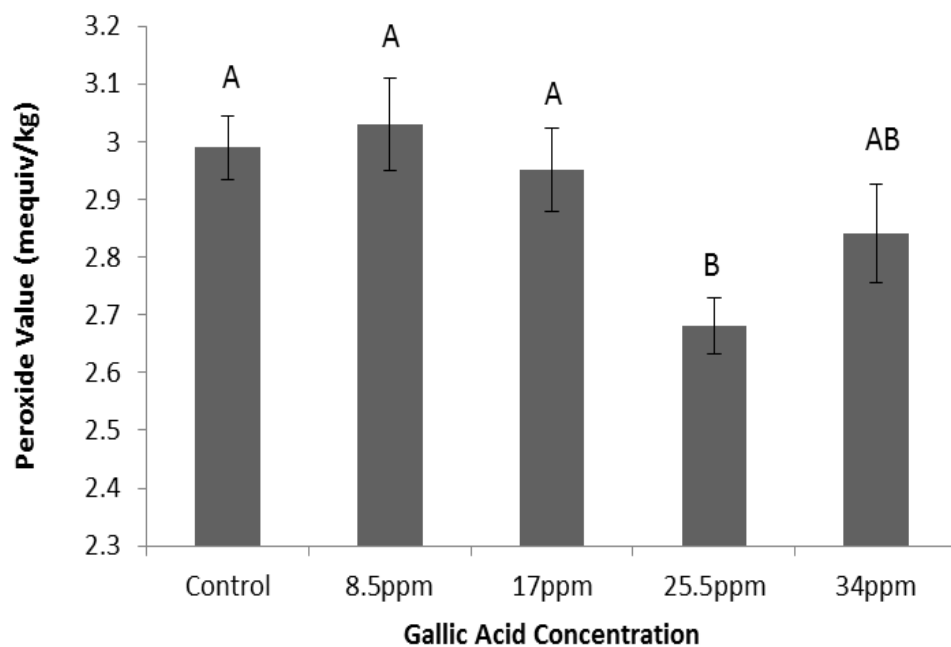
**Figure 6a – Effect of gallic acid concentration on total CLA yield in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



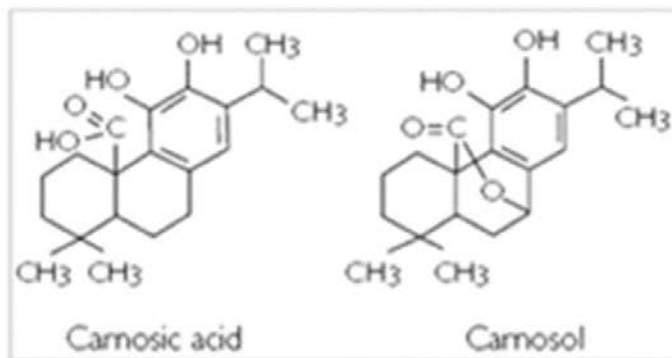
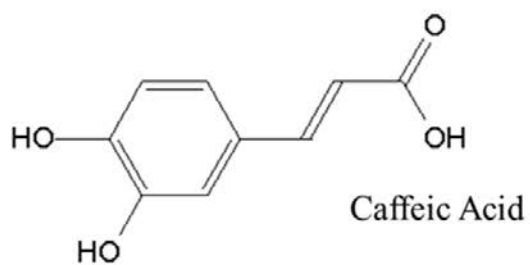
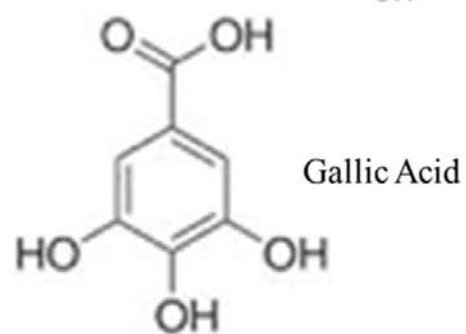
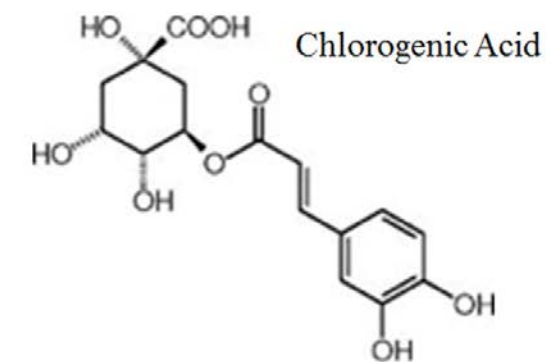
**Figure 6b - Effect of caffeic acid concentration on CLA isomer distribution in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



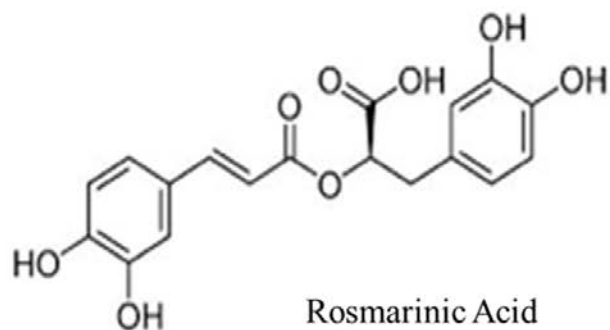
**Figure 6c - Effect of gallic acid concentration on peroxide value in refined, bleached deodorized soy oil with 0.35% iodine and UV light irradiated for 12 hours**



**Figure 7 – Antioxidant Structures**



Rosemary Extract  
1:3 ratio of Carnosic acid to Carnosol



## **CONCLUSION AND FUTURE RESEARCH**

Chlorogenic acid at 11 ppm was the most effective of the selected phenolic compounds of those selected in ranges used in promoting CLA formation and serving as an effective antioxidant. The most ineffective compounds, GA and CA, were the most polar and used at lowest concentrations. A balance of polar and non polar characteristics at a critical concentration seem important to dissolve in the oil (non-polar characteristics) and serve as an antioxidant (polar characteristics). Further studies of the selected compounds under a common equimolar and ppm range could be conducted to better understand the interaction of concentration and molecular structure on both CLA yields and PV.

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